Modulation of Tau Isoforms Imbalance Precludes Tau Pathology and Cognitive Decline in a Mouse Model of Tauopathy

Highlights

- Htau mice show cognitive deficits and tau pathology with reduced PFC activity
- *In vivo* trans-splicing yields long-term modulation of tau 3R/4R isoform ratio
- Early balance of tau isoforms in the PFC reduces tau pathology in aged htau mice
- Tau reprogramming prevents cognitive impairment and deficits in neuronal firing

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In Brief

Tau isoform imbalances in humans lead to neurological disorders. Espíndola et al. show that *in vivo* reprogramming of tau mRNA by trans-splicing in adult transgenic mice corrects tau isoform imbalance, yielding reduced pathological markers and preventing the loss of key functions such as neuronal activity and cognitive performance.
Modulation of Tau Isoforms Imbalance Precludes Tau Pathology and Cognitive Decline in a Mouse Model of Tauopathy

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SUMMARY

The microtubule-associated protein tau regulates myriad neuronal functions, such as microtubule dynamics, axonal transport and neurite outgrowth. Tauopathies are neurodegenerative disorders characterized by the abnormal metabolism of tau, which accumulates as insoluble neuronal deposits. The adult human brain contains equal amounts of tau isoforms with three (3R) or four (4R) repeats of microtubule-binding domains, derived from the alternative splicing of exon 10 (E10) in the tau transcript. Several tauopathies are associated with imbalances of tau isoforms, due to splicing deficits. Here, we used a trans-splicing strategy to shift the inclusion of E10 in a mouse model of tauopathy that produces abnormal excess of 3R tau. Modulating the 3R/4R ratio in the prefrontal cortex led to a significant reduction of pathological tau accumulation concomitant with improvement of neuronal firing and reduction of cognitive impairments. Our results suggest promising potential for the use of RNA reprogramming in human neurodegenerative diseases.

INTRODUCTION

Tau is a microtubule-associated protein, enriched in neuronal axons, that regulates neurite outgrowth and axonal transport, among other functions (Arendt et al., 2016; Medina et al., 2016; Morris et al., 2011). Under pathological conditions, such as gene mutations, failures in post-translational processing or clearance (reviewed in Bodea et al., 2016), hyperphosphorylated insoluble tau accumulates in the neuronal soma. These tau deposits are the hallmark of several diseases, referred to as tauopathies, which include Alzheimer’s disease and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (reviewed in Spillantini and Goedert, 2013).

Human tau is encoded by the MAPT gene, comprising 16 exons that produce six different tau isoforms in the adult brain, by alternative splicing of exons 2, 3, and 10 (Andreadis, 2005). Alternative splicing of exon 10 (E10) gives rise to tau isoforms with three (3R) or four (4R) microtubule-binding repeats (Andreadis et al., 1992; Goedert et al., 1989), found in equal amounts in the normal adult human brain. Imbalances in tau 3R/4R ratio are known as a disease-causative phenomenon, initially based on genetic evidence from patients carrying mutations affecting E10 splicing (Hutton et al., 1998; Spillantini et al., 1998). So far, more than 50 mutations have been found in the MAPT gene (Qian and Liu, 2014; Spillantini and Goedert, 2013), with about one-third of them affecting E10 splicing and consequently the normal 3R/4R balance (Niblock and Gallo, 2012). The majority of these mutations favor E10 inclusion, leading to 4R increase (Spillantini and Goedert, 2013), but mutations yielding 3R excess were also linked with disease (Stanford et al., 2003), suggesting that tau isoform imbalance is detrimental regardless of whether the ratio shifts toward 3R or 4R (reviewed in Andreadis, 2012). Although their etiology is known, no treatment has become available for those tauopathies; yet, early correction of aberrant E10 splicing arises as a potential therapeutic strategy to stop the onset of disease.

We previously validated the spliceosome-mediated RNA trans-splicing (Rodríguez-Martín et al., 2005) as a procedure to modulate endogenous tau E10 inclusion in the mouse brain (Avale et al., 2013) and in human neurons in culture (Lacovich et al., 2017). Here, we used this strategy to investigate whether modulation of relative content in tau isoforms prevents the development of pathological phenotypes in the htau mouse (Andorf et al., 2017), a validated model of tauopathy. Htau mice carry a full-length human MAPT gene that produces the six human tau isoforms in the adult brain, where production of 3R isoforms are favored over 4R. It was suggested that such 3R > 4R content might underlie tau pathology and the associated phenotypes observed in aged htau mice, such as tau hyperphosphorylation.
To test this hypothesis, we used trans-splicing to enhance E10 inclusion into the medial prefrontal cortex of adult htau mice. Trans-splicing reduced the contents of pathological tau in htau mice, which showed normal electrophysiological and behavioral performance, suggesting that early modulation of 3R/4R tau isoforms ratio has a beneficial effect over the tauopathy phenotypes in this model.

**RESULTS**

**Reprogramming of 3R to 4R Tau Isoforms in the Prefrontal Cortex of Htau Mice**

Tau pathology was reported both in the cortex and hippocampus of htau mice (Andorfer et al., 2003; Polydoro et al., 2009). Yet we found the most prominent accumulation of pathological tau in the medial prefrontal cortex (mPFC) (Figure S1). Moreover, the phenotypes we observed in this model are consistent with prefrontal dysfunction, so we selected the mPFC to modulate the 3R/4R ratio and analyze the potential functional rescue.

To promote E10 inclusion into endogenous tau transcripts, we used a pre-trans-splicing molecule (PTM), delivered by a lentiviral vector, (LV)-PTM4R (Figure 1A), as previously described (Avale et al., 2013). The trans-splicing reaction yields a chimeric RNA containing E10+, which codes for a 4R tau isoform. Control mice were injected with an LV carrying the same construct but lacking the trans-splicing domain (LV-PTM4R-ΔTSD; Figure 1B). LV-PTMs were injected at 2–3 months of age, before the onset of pathological tau accumulation. Behavioral, electrophysiological, and biochemical analyses were performed in 10- to 12-month-old mice (Figure 1C).

Endpoint RT-PCR (Figure 1D) was performed to visualize tau mRNA isoforms using primers spanning exons 9–13 of the tau transcript. As previously reported (Avale et al., 2013), htau mice produce very low amounts of E10+, which increased after PTM4R administration (htau-PTM4R). To perform a quantitative analysis of tau mRNA isoforms, real-time qPCR was performed after laser-captured microdissection of the mPFC co-injected with LV-PTMs and a fluorescent reporter (LV-GFP; Figure 1E). Moreover, the phenotypes we observed in this model are consistent with prefrontal dysfunction, so we selected the mPFC to modulate the 3R/4R ratio and analyze the potential functional rescue.

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Total amounts of hyperphosphorylated tau were also measured by western blotting in PFC homogenates of aged htau-PTM4R, htau-control, and WT mice (Figures 2E and 2F). A significant increase was observed in htau-control mice compared with WT mice, either at Ser 202 (Figures 2E and 2D) or at Thr231/Ser235 (Figure 2F), while htau-PTM4R mice showed values similar to WT mice (Figures 2E, 2F, and 2D).

We also quantified tau protein levels in soluble and insoluble protein fractions obtained from PFC homogenates by sarkosyl fractionation. Htau-control mice showed high amounts of insoluble tau (Figure 2G), yielding a 4-fold increase in the insoluble/soluble ratio compared with WT (Figure 2H), while htau-PTM4R mice showed a significant reduction in the insoluble tau fraction (Figure 2G), leading to a soluble/insoluble tau ratio similar to control WT mice (Figure 2H). Taken together, these results indicate that early local modulation of tau 3R/4R balance prevents the accumulation of insoluble/hyperphosphorylated tau in the PFC of aged htau mice.

**Electrophysiological and Behavioral Phenotypes of Htau-PTM4R Mice**

To assess whether a functional recovery could be achieved because of tau isoform modulation in the PFC of htau mice, we characterized the neuronal firing in aged mice, recording the electrophysiological activity of putative pyramidal neurons. Three tetrodes were placed in the mPFC of anesthetized mice, either htau-control, htau-PTM4R, or WT group, and extracellular unit activity was analyzed according to published criteria (de Almeida et al., 2013). Normal neuronal activity was disrupted in the htau-control group, which displayed a significant decrease in mean firing rate compared with age-matched controls (Figures...
Figure 1. Local Reprogramming of 3R to 4R Tau Isoforms in the Prefrontal Cortex of Htau Mice

(A and B) Map of lentiviral vectors. (A) LV-PTM4R used for trans-splicing and the expected chimeric transcript containing E10. (B) Lentiviral vector LV-PTM4R ΔTSD used for control mice.

(C) Time course of the experiments performed in the study.

(D) Analysis of tau isoforms by endpoint RT-PCR with primers spanning E9 and E13.

(E) Laser-capture microdissection of the mPFC of htau mice co-injected with LV-PTM and an LV carrying GFP.

(F) Relative content of tau isoforms determined by RT-qPCR with specific primers for 3R (E10–) and 4R (E10+). Data are expressed as the isoform ratio; mean ± SEM; *p < 0.05, two-tailed t test; n = 4 per group.

(G) Total tau mRNA, showing no differences among treatments.

(H and I) Western blot detection of tau protein contents in PFC homogenates. (H) Tau isoforms 3R and 4R contents and (I) total tau protein, related to actin loading control. Mean ± SEM; **p < 0.01 and ***p < 0.001, two-tailed t test (WT = 3, htau-control = 4, and htau-PTM4R = 5, htau Ni = 3). Full blots are shown in Figure S2A.
Remarkably, balancing 3R/4R tau isoforms in htau-PTM4R mice resulted in a recovery of neuronal activity, with a mean firing rate similar to the observed WT mice (Figures 3B and S3C). Furthermore, the population of active neurons firing over 4 Hz was decreased in htau-control mice compared with WT (WT, 21.4%; htau, 7.7%; chi-square p = 0.034) and was recovered in hTau-PTM4R (WT, 21.4%; htau-PTM4R, 25.5%; chi-square p = 0.59) (Figure 3B).

We next analyzed cognitive performance in the novel object recognition (NOR) test, a task known to rely on mPFC integrity (Morici et al., 2015), which is severely impaired in htau mice (Castillo-Carranza et al., 2014; Polydoro et al., 2009). As previously reported, aged htau-control mice exhibited poor recognition performance, showing a discrimination index of 50%, compared with the 80% observed in the WT group (Figure 3C). Interestingly, htau-PTM4R mice showed similar performance as WT to discriminate the novel object (Figure 3C), demonstrating full recovery on this task. The time spent in exploration was similar between groups (Figure 3D). Yet an open-field analysis revealed a small increase in total locomotion in htau-control mice compared with WT (Figure 3E), with more activity in the center of the arena (Figure 3F). However, these phenotypes were only partially rescued in the htau-PTM4R group, showing no differences from WT and htau-control groups. On the other hand, when tested in the elevated plus maze, htau-control mice displayed about 50% of entries into open arms (Figures 3G and 3H), suggesting no preference for protected arms, while htau-PTM4R mice showed 33% entries, similar to WT mice. However, no statistically significant differences were observed between groups on this task. Moreover, in our hands the htau model did not

Figure 2. Hyperphosphorylated and Insoluble Tau Contents in the PFC

(A–D) Hyperphosphorylated tau detected by immunohistochemistry using CP13 (S202) (A and B) and AT180 (T231/S235; C and D) antibodies in coronal sections spanning the mPFC. (B and D) Quantification of positive cells in htau-control and htau-PTM4R. CP13, n = 3 per group; AT180, n = 4 per group; two to four sections per mouse; mean ± SEM; **p < 0.01 and ***p < 0.001, two-tailed t test.

(E and F) Hyperphosphorylated tau protein contents determined by western blot in PFC homogenates using CP13 (E) in WT (n = 4), htau-control (n = 6), and htau-PTM4R (n = 6) or (F) AT180: WT (n = 3), htau-control (n = 4), and htau-PTM4R (n = 7). Mean ± SEM; *p < 0.05, one-way ANOVA followed by Tukey’s post hoc test. Full blots are shown in Figure S2D.

(G and H) Sarkosyl insolubility assay. Soluble and insoluble fractions obtained from PFC homogenates were immunoblotted (G) to quantify total tau in each fraction (h). WT, n = 2; htau-control, n = 4; htau-PTM4R, n = 3; mean ± SEM; *p < 0.05, one-way ANOVA followed by Tukey’s post hoc test.

3A, 3B, S3C, and S3D). Remarkably, balancing 3R/4R tau isoforms in htau-PTM4R mice resulted in a recovery of neuronal activity, with a mean firing rate similar to the observed WT mice (Figures 3B and S3C). Furthermore, the population of active neurons firing over 4 Hz was decreased in htau-control mice compared with WT (WT, 21.4%; htau, 7.7%; chi-square p = 0.034) and was recovered in hTau-PTM4R (WT, 21.4%; htau-PTM4R, 25.5%; chi-square p = 0.59) (Figure 3B).
show impairments in spatial memory assessed in the Morris water maze test; both control and htau-PTM4R mice performed similarly to WT mice (Figures S3A and S3B). Together, electrophysiological and behavioral analyses indicate that the local early modulation of tau isoforms in htau mice prevents impairments in PFC functionality and restores normal performance in the NOR task.

**DISCUSSION**

In this study we show that modulation of 3R/4R tau isoform balance in the prefrontal cortex precludes tau pathology and related phenotypes in the htau mouse model of tauopathy.

The use of mouse models to analyze tau isoform imbalance is challenging, because 4R is the only isoform present in the WT adult mouse brain. By contrast, the htau model bears a full-length normal human MAPT transgene that, despite the lack of mutations, favors E10 exclusion and produces an excess of 3R isoform. This might be due to the lack of specific splicing regulatory elements in the mouse neurons (or a different combination of splicing factors), which impairs the correct processing of the human tau transcript. Thus, compared with either the adult human brain expressing both isoforms in an equimolar ratio or with WT adult mouse brain, which expresses 4R tau only, the htau brain has an aberrant content of tau isoforms that correlates with tau pathology (Andorfer et al., 2003). Moreover, tau pathology is not likely to be due to tau overexpression in this model, as the same transgene in a WT background does not recapitulate tauopathy (Duff et al., 2000), suggesting that endogenous murine 4R tau might balance the excess of human 3R tau. These features make the htau model particularly interesting to test in vivo strategies that modulate E10 splicing and assess the phenotypic consequences of such modulation.

We have demonstrated herein that by increasing E10 inclusion, we reached a balanced 3R/4R ratio in the mPFC of htau-PTM4R mice, which prevents the pathological phenotypes observed under 3R excess in htau-control mice. It is noteworthy that trans-splicing strategy reduces tau pathology without changes in the total amount of tau protein compared with control htau, and creating a ∼1.0 3R/4R ratio of human tau in adult mice is not detrimental. Indeed, in our conditions, the increase of the 4R isoform by means of the trans-splicing strategy did not induce tau pathology; instead, consistent rescue was observed in histological, functional, and behavioral analyses. Strikingly, enhancing 4R isoform by intraventricular ASO treatment was reported to induce tau pathology and seizures in young htau mice (Schoch et al., 2016). However, that study was performed at 4 months of age, before the onset of the phenotypes we and others analyzed in the htau model (Andorfer et al., 2003; Castillo-Carranza et al., 2015; Polydoro et al., 2009). On the other hand, ASO intracerebroventricular (i.c.v.) administration likely drives global 4R increase in the whole brain, which clearly differs from our strategy that locally targets a specific affected brain area. Our present results, in context with previous reports using this model, suggest that the long-term 3R > 4R imbalance might underlie tau pathology, particularly prefrontal dysfunction, in aged htau mice, which can be prevented by balancing the 3R/4R ratio at an early stage.
We have shown here that trans-splicing reprogramming of the endogenous tau transcript in vivo is a suitable strategy to achieve a phenotypic recovery. Our previous work (Lacovich et al., 2017) highlights the feasibility of this strategy to modulate tau isoform balance in human neurons, independent of the predominant isoform. Furthermore, RNA reprogramming can also correct missense mutations inside E10 (Rodriguez-Martin et al., 2009), without changing the total levels of tau protein. In this context, tau RNA reprogramming by trans-splicing arises as a versatile gene therapy approach with promising perspectives for human tauopathies. Further studies would be needed to optimize treatment conditions that ensure the most efficient therapeutics effects.

**EXPERIMENTAL PROCEDURES**

**Mice**
All animal procedures were designed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and protocols were approved by the Institutional Committee of INGEBI-CONICET (protocol number 005/2016) and FCeYN, University of Buenos Aires. Mice were housed under a 12 hr dark/light cycle with ad libitum access to food and water. Htau transgenic mice were obtained from Jackson Laboratories (stock number 005491) and bred to obtain Htau and WT colonies. All mice were genotyped as previously described (Andorfer et al., 2003). Male adult mice were used to conduct all the experiments described in this study.

**LVs**
Tau PTMs were delivered into LVs, prepared as detailed previously (Avale et al., 2013) and in Supplemental Experimental Procedures.

**Stereotaxic Injections**
Male mice aged 8–10 weeks (weight 25–30 g) were bilaterally injected with 2 µL of lentiviral suspension (0.6 x 10^5 TU/mL) into the PFC at the following coordinates (Paxinos and Franklin, 2011): anteroposterior, +2.5 mm; lateral, ± 0.5 mm; and dorsoventral, −2.5 and −1.5 mm, measured from the bregma. Surgery was performed under the protocol detailed in Supplemental Experimental Procedures.

**Laser-Capture Microdissection**
Fluorescent areas were cut out of a series of eight to ten sections using the ArcturusXT laser-capture microdissection system (Thermo Fisher Scientific) by infrared (IR) laser energy combined with a UV cutting laser (Supplemental Experimental Procedures). Total RNA was isolated from the collected tissue (0.5–1 mm³), yielded 0.5–1 µg RNA per sample.

**Detection of Tau mRNA Isoforms**
Total RNA was extracted using the RNeasy Lipid Tissue Kit (QIAGEN) or, in laser-capture microdissection (LCM)-dissected tissue, with the PicoPure RNA extraction Kit (Thermo Fisher Scientific). Reverse transcription was performed using TaqMan RT (Applied Biosystems) with 0.5 µg of RNA and an equimolar ratio of oligo(dT) and random hexamers. Endpoint PCR was performed with primers spanning exons 9–13, as previously described (Avale et al., 2013). Human 4R, 3R, and total tau mRNA isoforms were detected using real-time PCR with specific pairs of primers for each isoform either spanning E9–E10 or E9/11–E11, respectively, or for all human tau isoforms spanning at least E7–E8 (Supplemental Experimental Procedures).

**Immunohistochemistry**
Phosphorylated tau was detected on free-floating coronal sections (40 µm) with either CP13 (1:100) or AT180 (1:500) (Thermo Fisher Scientific) monoclonal antibodies and developed with 3,3'-diaminobenzidine (DAB) (Sigma-Aldrich). Positive cells were quantified in the PFC between +1.54 and +2.5 mm from bregma.

**Protein Extraction and Western Blotting**
PFC homogenates were separated on 12% SDS-polyacrylamide gels, transferred, and blotted using antibodies directed against 3R tau (1:2,000 anti-tau 3-repeat isoform RD3; mouse monoclonal; Millipore), 4R tau (1:1,000 anti-tau 4-repeat isoform RD4; mouse monoclonal; Millipore), total tau (1:10,000 rabbit polyclonal; Dako), phospho-Ser202 (CP13 monoclonal, 1:300), AT-180 (1:1,000; Thermo Fisher Scientific), or mouse β-actin (mouse monoclonal, 1:10,000; Abcam). See details in Supplemental Experimental Procedures.

**Sarkosyl Insolubility Assay**
Fractionation of soluble and insoluble proteins was performed as previously described (Noble et al., 2009; Andorfer et al., 2003). Briefly, brains were homogenized, incubated with 1% sarkosyl reagent (Sigma-Aldrich), and ultracentrifuged to obtain soluble (supernatant) and insoluble (pellet) fractions (Supplemental Experimental Procedures). Total tau was detected on each fraction by western blot using total tau (1:10,000, rabbit polyclonal; Dako).

**Electrophysiological Recordings**
In vivo electrophysiological experiments we conducted as described previously (6e Almeida et al., 2013). See Supplemental Experimental Procedures.

**Behavioral Tests**
All mice tested were sibling cohorts of males aged 10–12 months. Experiments were performed between 13:00 and 17:00 under dim illumination, in a separate behavioral room, where mice were transferred in advance. Behavioral experiments were analyzed by double-blinded operators.

**Elevated Plus Maze**
This test was performed as described previously (Avale et al., 2004). See details in Supplemental Experimental Procedures.

**Novel Object Recognition**
The protocol was performed similarly to that previously described (Polydoro et al., 2009), with minor modifications following a well-described methodological procedure (Leger et al., 2013). Briefly, in the training phase mice were allowed to explore identical objects for 10 min, and 5 hr later, mice were tested for 5 min in the same chamber with a familiar and a novel object. Discrimination index was calculated as the time spent exploring the novel object divided by the total time spent exploring both objects (familiar and novel). See details in Supplemental Experimental Procedures.

**Statistical Analyses**
Data were analyzed using GraphPad Prism. When normal distribution was assumed, the three experimental groups were analyzed using one-way ANOVA, followed by Tukey’s test. When indicated, paired comparisons were done using two-tailed Student’s t test. When normal distribution could not be assumed, the Kruskal-Wallis non-parametric test was used to compare groups.

**SUPPLEMENTAL INFORMATION**
Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.03.079.

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AUTHOR CONTRIBUTIONS
S.E., A.D., R.J.A., and M.S. conducted experiments and analyzed data. J.E.B. and J.E.F. supervised experiments and analyzed data. J.-M.G. contributed materials and expertise. M.E.A designed the project, supervised experiments, and wrote the paper.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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